AWARD NUMBER: W81XWH-12-1-0490

TITLE: : Identifying Neurobiological Markers of the Broader Autism Phenotype

PRINCIPAL INVESTIGATOR: Professor Sarah Wilson

CONTRACTING ORGANIZATION: University of Melbourne
                                         Parkville, Australia 3052

REPORT DATE: January 2015

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. Army Medical Research and Materiel
                            Command Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
                                              Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
January 2015	Final	30 Sep 2012 - 19 Oct 2014

4. TITLE AND SUBTITLE
: Identifying Neurobiological Markers of the Broader Autism Phenotype

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-12-1-0490

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Professor Sarah Wilson, Professor Ingrid Scheffer, Miss Valerie Yap

E-Mail: sarahw@unimelb.edu.au, i.scheffer@unimelb.edu.au, v.yap2@student.unimelb.edu.au

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Melbourne
Jessica Piesse (Contractor)
Grattan St
Parkville VIC 3052
Australia

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command

Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Our project was designed to identify more precise and objective, performance-based measures of the Broader Autism Phenotype (BAP). In particular, we focused on the social communication difficulties commonly experienced by people with the BAP using sophisticated techniques that we developed to capture the acoustic properties of speech important for emotional expression. We have investigated this issue in detail in individuals with and without the BAP using a range of tasks measuring skills important in emotional and social interactions. This project also maps the brain systems that underpin social communication in the BAP by using state-of-the-art brain imaging techniques that measure brain structure and function. Our study design included linking these behavioral and brain findings to gain a more complete understanding of the BAP.

To achieve the study aims, we developed a novel neuroimaging protocol to measure the brain systems that underpin social communication in the BAP, as well as an interactive, web-based computer program designed for efficient collection of behavioral data, including capture of the acoustic properties of speech important for emotional expression. Using these new protocols (Experiments 1 & 2), we have successfully collected data for project participants, the methodology and results of which are presented in this report. Final data analyses for Experiments 1 and 2 are currently underway, with published outcomes expected within the next 12 months. Of particular note, our functional neuroimaging data shows associations between vocal emotion processing and neuronal activation within the mirror neuron and limbic systems, in accordance with our research hypotheses. These findings have important implications for understanding social communication difficulties in the BAP. High-impact scientific journals have been targeted for the publication of our results and the data will be presented at national and international scientific research meetings over the coming 12 months. Moreover, between 2013-2014, our research has been presented at five scientific research meetings.

15. SUBJECT TERMS
Broader Autism Phenotype (BAP), Autism Spectrum Disorder (ASD), Social Communication, Vocal Emotion Processing, Functional Magnetic Resonance Imaging (fMRI), Structural MRI

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	**b. ABSTRACT** Unclassified	**c. THIS PAGE** Unclassified	Unclassified	23	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

Page

INTRODUCTION

Our highly innovative project was designed to identify more precise and objective, performance-based measures of the Broader Autism Phenotype (BAP) to advance scientific investigation of the causes and treatment of Autism Spectrum Disorders (ASD). The BAP is a milder phenotypic variant of ASD that primarily involves social-relatedness difficulties. Family members with the BAP are likely to be carriers of one or more ASD susceptibility genes, providing an ideal model for identifying genes underpinning impaired social behavior. Thus, our study focused on the social communication difficulties commonly experienced by people with the BAP using new and sophisticated techniques that we developed to capture the acoustic properties of speech important for emotional expression. We investigated this issue in detail, both in individuals with and without the BAP, using a range of tasks measuring skills important in emotional and social interactions. This project also maps the brain systems that underpin social communication in the BAP by using state-of-the-art brain imaging techniques that measure brain structure and function. Our study design included linking these behavioral and brain findings to gain a more complete understanding of the BAP.

Our novel approach involved assessing people from rare large families, in which there are many members with either autism or the BAP with these social communication difficulties. This allows complex genetic patterns to be traced in these families, so that we can assess how the social-relatedness difficulties associated with autism and the BAP are inherited across generations. This is a highly effective way to find dominant genes causing social difficulties in autism and the BAP. In this way, the current project not only changes how autistic traits are viewed, as falling along a spectrum that blends with the normal population, but it also provides new neuroscientific insights into the changes in the brain that underpin autistic behavior. This project therefore lays the vital foundations for the development of targeted treatments specifically designed to ease the profound social difficulties than can be experienced by people with ASD and their families.

Keywords: Autism Spectrum Disorders (ASD), Broader Autism Phenotype (BAP), nonverbal emotions, social cognition, structural brain imaging, functional brain imaging, endophenotype

OVERALL PROJECT SUMMARY

The research objectives of our project included performing a detailed behavioral analysis of emotional identification, imitation, and expression using our new audiovisual technologies to fully characterize the psychoacoustic endophenotype of the BAP. We also linked this endophenotypic profile to measures of pragmatic language, empathy and social cognition. We then used advanced functional and structural neuroimaging to investigate differences in the neural correlates of the perception and production of emotionally expressive speech in BAP participants and healthy controls, with a view to identifying neural endophenotypic markers, and determining the relationship between the neural and psychoacoustic endophenotypes, as well as their sensitivity and specificity to the BAP. By addressing these objectives our project provides clinicians with innovative neurobehavioral assessment tools for precise characterization of the BAP that complement existing clinical methods for diagnosis, as well as contributing to our understanding of the neurobiological basis of emotional expression in the BAP, ultimately contributing to molecular genetic analyses to discover ASD genes.

In light of these objectives, the information below describes our research accomplishments, listed according to each milestone described in our approved Statement of Work (effective start date: April 19, 2013). Methodology for our behavioral and functional neuroimaging paradigms is also presented, along with a summary of our findings.

Milestone #1: Regulatory review and approval for human study

Local IRB (Royal Children's Hospital, Human Research Ethics Committee) approval to conduct the study was granted on July 23, 2012. Reports for annual continuing review were subsequently submitted and approved by our local IRB between 2012-2014.

Milestone #2: Protocol and technique

An interactive web-based computer program was purpose-built and then extensively piloted to collect data for the specific tasks included in our behavioral paradigm to measure social communication difficulties in the BAP. Among other things, this web-based program incorporates a range of vocal sound files of varying nonverbal emotions to which participants respond. It also uses highly innovative, purpose-built voice recording software to collect participant samples of emotions. It has been effectively applied to collect behavioral data from study participants, with this data now undergoing final analyses using purpose-developed computational algorithms to characterize the psychoacoustic endophenotype of the BAP. This data is targeted for publication in a high impact, international scientific journal over the next 12 months. The novel web-based methodology developed for our behavioral paradigm and the findings from this work to date are described on pages 6-10 of this report.

A set of in-scanner fMRI tasks that produce behavioral responses equivalent to the out-of-scanner behavioral paradigm was also purpose-developed and extensively piloted. This ensured that patterns of brain activation collected in-scanner can be accurately correlated with out-of-scanner behavioral performance to ensure cognitive and emotional processes of interest are effectively captured. The paradigm employs state-of-the-art structural and functional MRI techniques, for which our research team is recognized as leading the field. Combined, the behavioral and neuroimaging paradigms represent sophisticated research tools that have been successfully used with the study participants. Analyses of the neuroimaging data as well as statistical correlations between the behavioral and neuroimaging data are currently underway, and are again targeted for publication in high impact, international scientific journals over the next 12 months. The novel methodology developed for our functional neuroimaging paradigm and the findings from this work to date are described on pages 11-16 of this report.

Milestone #3: Participant ascertainment

As planned, family members from the rare large families with multiple individuals diagnosed with either ASD or the BAP have been assessed with the Family History Interview as well as an extensive range of cognitive and behavioral measures to determine BAP status. The social discourse of these family members has also been precisely analyzed using purpose-written auditory software, to identify individuals with and without the psychoacoustic marker of the BAP. Control participants matched for age, sex and IQ with no history of neurologic, psychiatric or hearing disorders have also been successfully identified and assessed with the study measures.

Milestone #4: Publication of behavioral findings and presentation of results at international conferences

Behavioral data has been successfully collected from project participants using our purpose-built web-based computer program incorporating various emotion-related tasks, including stimuli from the Montreal Affective Voices (MAV; Belin, Fillion-Bilodeau, & Gosselin, 2011). Analyses of the behavioral data using IBM SPSS Statistics are being finalized, with published outcomes expected within the next 12 months, targeting high impact scientific journals. Conference abstracts will also be submitted to national and international community and scientific meetings, including the International Meeting for Autism Research (IMFAR), to ensure that our behavioral findings are widely disseminated. For all publications and presentations the funding support of the US Department of Defense will be appropriately recognized and acknowledged.

Behavioral Methodology and Findings

Our web-based computer program (URL: http://emotions.sollysweb.com/index_new.php) incorporated a range of emotion-related tasks to measure our participants' ability to spontaneously produce, recognize and imitate nonverbal vocal emotional expressions belonging to the six basic emotions (i.e., anger, fear, disgust, surprise, happiness, sadness). Other measures of general auditory processing ability were also included in our program to account for individual differences in pitch perception, which may confound performance on the emotion-related tasks. To maintain participant motivation, throughout the program participants were presented with a "progress bar/circle" to keep track of the number of items/tasks they were yet to complete. Participants completed all tasks alone in an anechoic chamber. Voice recordings were collected in WAV format (sampling frequency: 44.1 kHz) using a high quality digital microphone, and auditory stimuli were presented from an external subwoofer speaker at a comfortable volume (sound intensity: approximately 70 dB). All data collected with this computer program were stored on a secure, password-protected web-based server.

Emotion Production Task
Figure 1 shows a typical trial from the Emotion Production Task. In each trial, participants were presented with one basic emotion to express with their voice. Of note, participants were first asked to imagine that they were feeling the emotion before expressing it vocally. Across trials, participants were explicitly advised not to use words (i.e., nonverbal) to express each emotion (e.g., "No!" "Hey!" "Wow!" "Yay!") in order to minimize the presence of interactions between emotional and linguistic properties of such vocalizations when analyzing acoustic markers in the data.
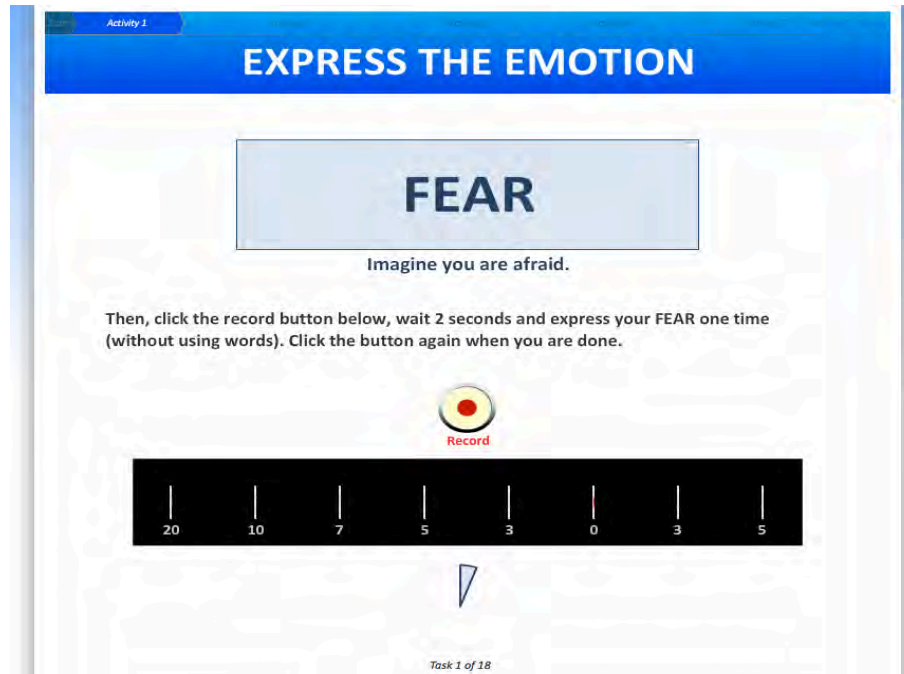
Figure 1. Example of a typical trial in the Emotion Production Task from the web-based computer program. In this trial, the participant is presented with the basic emotion of "Fear" and instructed to imagine that they are feeling afraid before producing a fearful expression with their voice. The participant then clicks the "Record" button to record their expression and can refer to the black bar to monitor microphone levels. The progress bar at the top of the screen indicates that the participant has five additional tasks to complete (mix of emotion-related tasks and tasks of general sound processing), while the progress circle at the bottom shows the number of trials remaining (17) in this particular task.

Participants completed a total of 18 trials, over which they were required to express the same basic emotion across three consecutive trials. Data from this task is being analyzed with purpose-built statistical algorithms to determine the specific acoustic properties of the six basic emotions. Figure 2 shows waveforms and spectrograms of nonverbal vocal emotional expressions belonging to each of the six basic emotions, as produced by male and female participants.
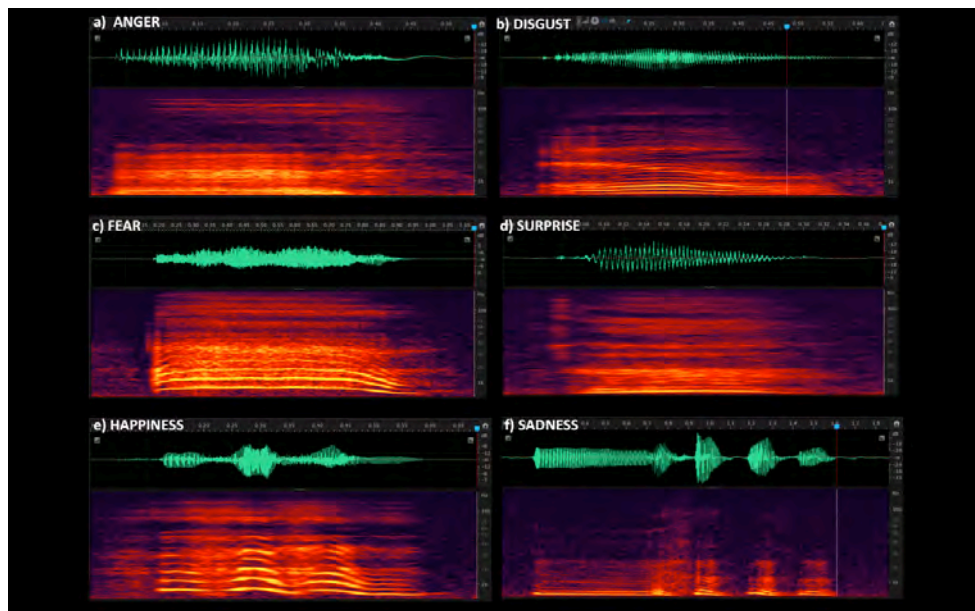

Figure 2. Waveforms (top image) and spectrograms (bottom panel) from spontaneous nonverbal vocal emotional expressions belonging to the six basic emotions, as produced by male and female participants using the web-based Emotion Production Task.

Emotion Recognition Task

Figure 3 shows a typical trial from the Emotion Recognition Task. In each trial, participants were first presented with a vocal stimulus from the Montreal Affective Voices (MAV) task, then asked to identify the emotion in the voice, and to rate its intensity (1=weak emotion to 7=strong emotion). MAV stimuli with no emotional content (i.e., neutral voices, rating of 0) were also presented as a control task. Participants were required to make their responses by clicking along the appropriate dimension of the visual wheel shown in Figure 3, or to click on the central circle (i.e., "No Emotion" category) to identify neutral voices.
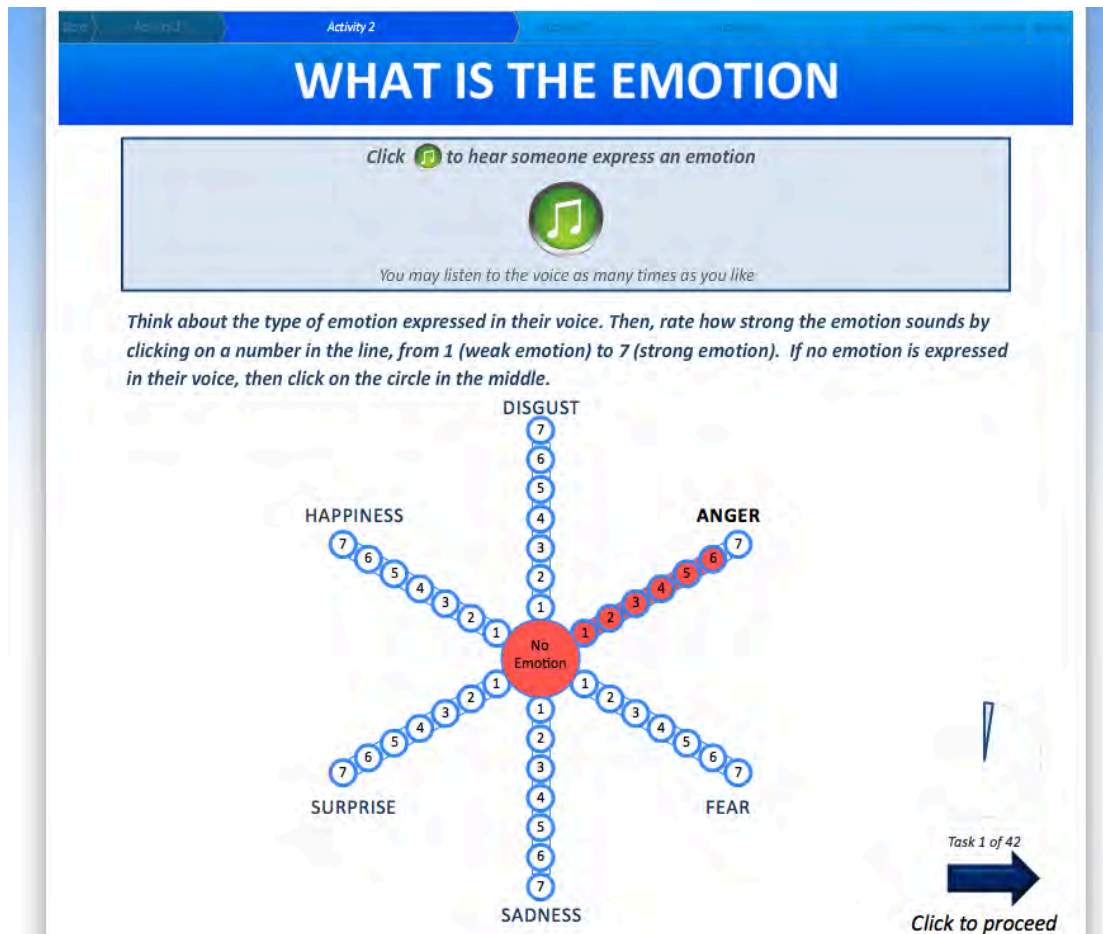

Figure 3. Example of a typical trial in the Emotion Recognition Task from the web-based computer program. Participants are instructed to click on the green button to hear a vocal stimulus (MAV), and to then identify the emotion in the voice and rate its intensity using the visual wheel. Here, the participant has clicked on the number "6" on the "ANGER" arm of the wheel to indicate that the MAV stimulus represents a relatively strong angry expression. The progress bar at the top of the screen indicates that the participant has four additional tasks to complete (mix of emotion-related tasks and tasks of general sound processing), while the progress circle at the bottom shows the number of trials remaining (41) in this particular task. Once completed, the participant then clicks the blue arrow to proceed to the next trial of the task.

Participants completed a total of 42 randomized trials, over which 6 MAV stimuli (3 male, 3 female voices) were presented from each of the six basic emotions and the "No Emotion" category. Figure 4 shows the distributions of accuracy scores and intensity ratings from the Emotion Recognition Task from a sample of control participants. As shown, the majority of control participants achieved high scores for percentage accuracy ($M=80.80$, $SD=7.385$), while more variation in the spread of control data was found for intensity ratings ($M=137.18$, $SD=24.5$).
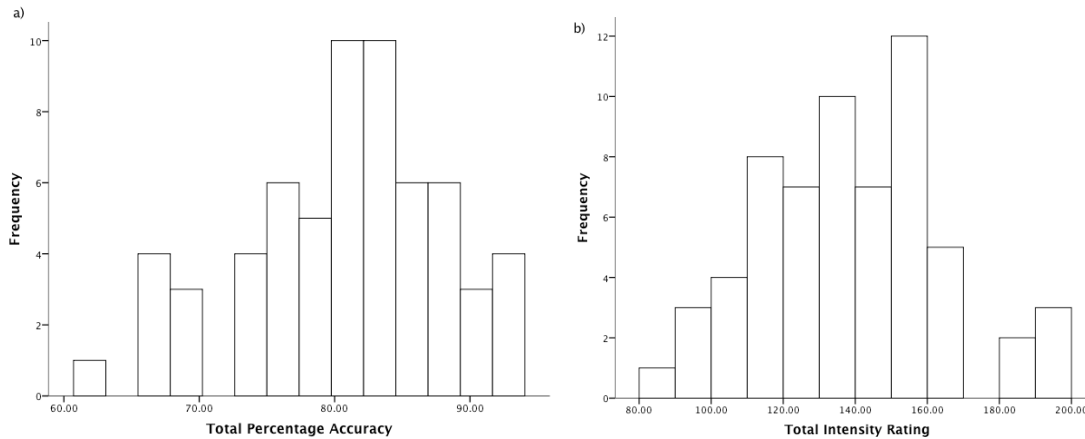
8

Figure 4. Histograms of the distribution of a) total percentage accuracy scores and b) total intensity ratings from a sample of control participants performing the web-based Emotion Recognition Task.

Emotion Imitation Task

Figure 5 shows a typical trial from the Emotion Imitation Task. In each trial, participants were presented with a vocal stimulus from the Montreal Affective Voices (MAV) task and informed about the type of emotion expressed in the voice. Participants were then required to imitate the speaker's emotional expression as accurately as possible. Gender-specific MAV stimuli were presented to each participant (e.g., male voices to male participants) to account for differences in acoustic properties across male and female voices.
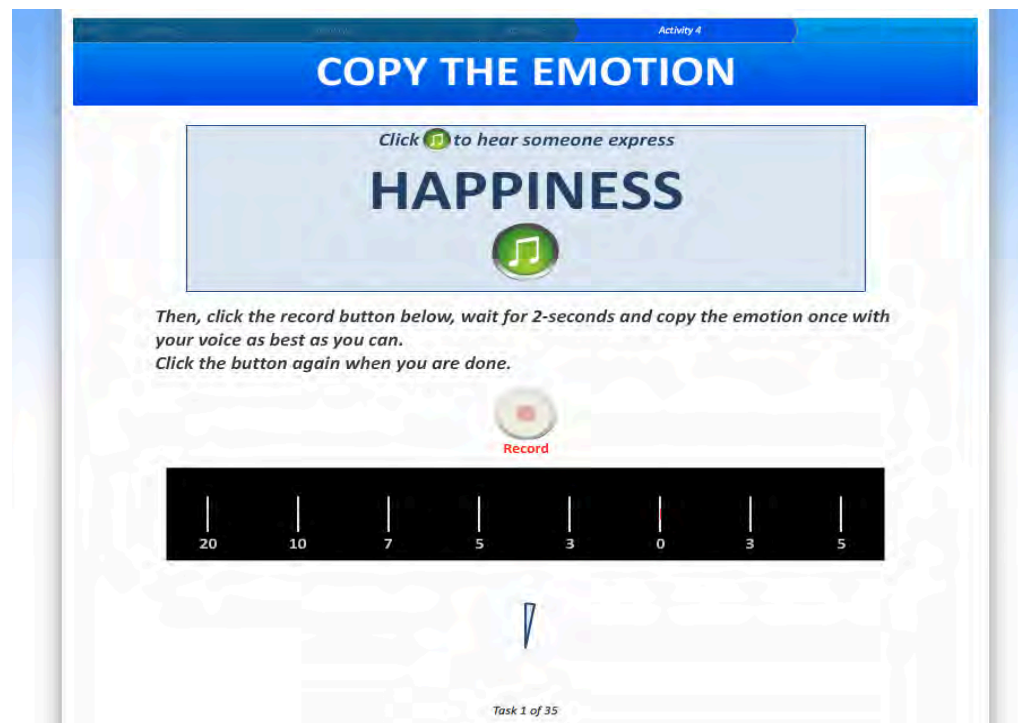

Figure 5. Example of a typical trial in the Emotion Imitation Task from the web-based computer program. In this trial, a participant is instructed to click the green button to hear a happy vocal expression (MAV stimulus) and to then imitate the happy expression as accurately as possible. As per the Emotion Production Task, participants then click the "Record" button to record their expression and can refer to the black bar to monitor microphone levels. The progress bar at the top of the screen indicates that the participant has two additional tasks to complete (mix of emotion-related tasks and tasks of general sound processing), while the progress circle at the bottom shows the number of trials remaining (34) in this particular task.

9

Participants completed a total of 35 randomized trials, over which five gender-specific MAV stimuli were presented from each of the six emotions and the "No Emotion" category. Data from this task are being analyzed with purpose-built statistical algorithms, which compare specific acoustic properties between the participants' vocal expressions and vocal expressions from the MAV task to determine the accuracy of imitation. Figure 6 shows waveforms and spectrograms of MAV stimuli templates belonging to each of the six basic emotions, along with imitations made by a male subject participating in the study.
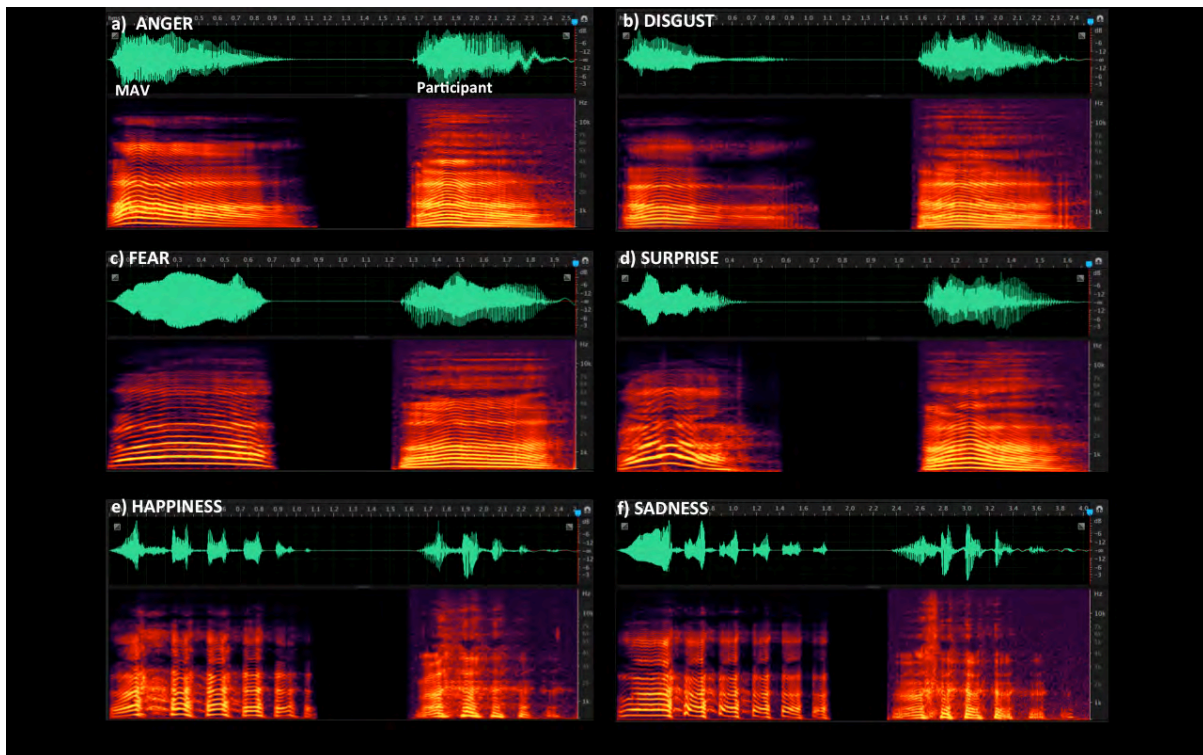

Figure 6. Waveforms (top panel) and spectrograms (bottom panel) of male MAV stimuli for each of the six basic emotions and the corresponding vocal responses produced by a single male participant performing the web-based Emotion Imitation Task. Here, MAV stimuli and their corresponding imitations are combined into a single sound file for each of the six basic emotions.

Milestone #5: Publication of structural and functional neuroimaging results and presentation of results at international conference

Neuroimaging data has been successfully collected from project participants using our state-of-the-art functional and structural techniques. Analyses of the neuroimaging data using both statistical parametric mapping (SPM) software and our in-house techniques are currently being finalized, with published outcomes expected within the next 12 months targeting high impact scientific journals. Published outcomes will include the relationship between neural and psychoacoustic endophenotypes of the BAP, as well as their sensitivity and specificity to the BAP. These findings will also be submitted for presentation at national and international community and scientific meetings, including the International Meeting for Autism Research (IMFAR). Again, for all publications and presentations the funding support of the US Department of Defense will be appropriately recognized and acknowledged.

Functional Neuroimaging Methodology and Findings

Participants performed two tasks of emotion processing within a 3-Tesla Siemens MRI scanner. These tasks were designed to identify activity in brain regions underpinning the production and recognition of nonverbal vocal emotional expressions belonging to the six basic emotions (i.e., anger, disgust, fear, happiness, sadness, surprise). Participants received prior training for the in-scanner tasks on a computer program in a mock scanner environment to increase the reliability of the results. The brain images presented below (Figures 9 & 12) show that the functional neuroimaging paradigm successfully activated key brain regions associated with emotion processing, including the hypothesized mirror neuron system (MNS), which underlies social cognitive skills such as the ability to empathize with others. As described in our initial project proposal, our neuroimaging paradigm has therefore successfully allowed associations between MNS functioning and social communication ability to be examined, contributing important scientific knowledge about the neurobiology and genetic basis of social behavior in ASD.

Task 1: Vocal Emotion Production

Whilst in-scanner, participants were required to make nonverbal vocalizations belonging to two primary categories: (1) vocal expressions of the six basic emotions (Emotion Condition), and (2) non-emotional vocalizations (Neutral Condition). Figure 7 shows the sequence of events in a typical trial from each condition. In the Emotion Condition, emotion labels (i.e., "Anger", "Disgust", "Fear", "Happiness", "Sadness", "Surprise") were presented visually across trials. Participants were instructed to imagine that they were feeling the emotion presented and then to express the emotion vocally without using words. In the Neutral Condition, the label "No Emotion" was presented visually across trials, and participants were instructed to produce a stereotyped monotonous vocal response, which they had practised with the experimenter prior to scanning. Across conditions, participants were instructed and had practised producing vocalizations with minimal facial and body movement.
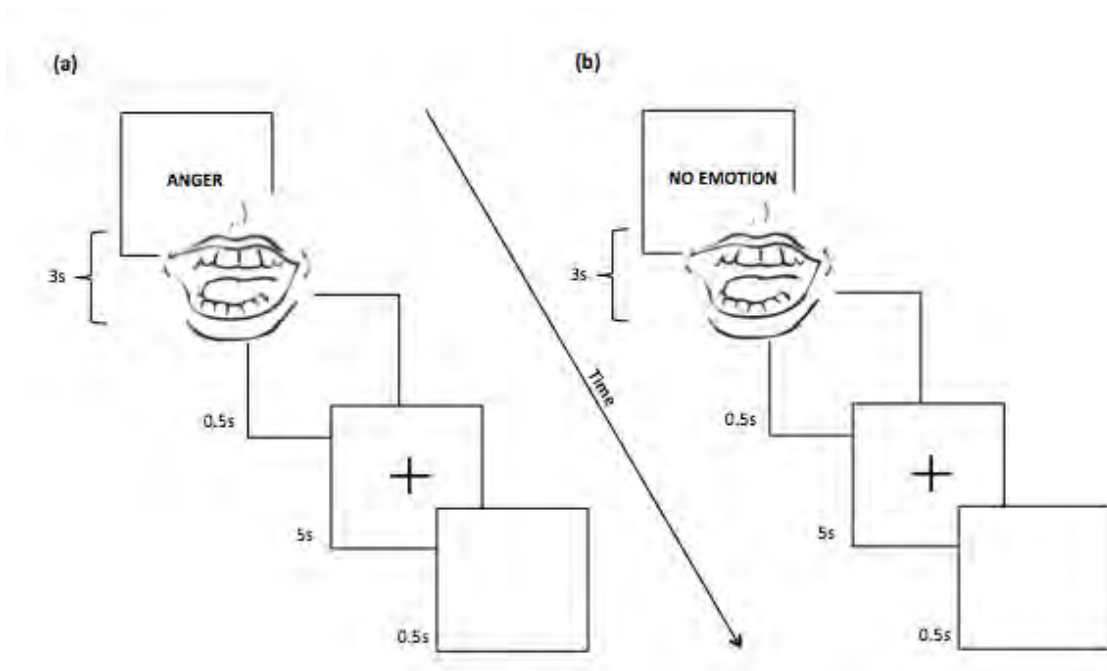

Figure 7. Sequence of events in a single trial from the (a) Emotion Condition and (b) Neutral Condition in Task 1. In the Emotion Condition trial shown here, the emotion label "Anger" is presented for 3s, during which the participant is required to imagine feeling angry and to make an angry vocal expression. After the offset of the label, a fixation cross is presented for 5s. In the Neutral Condition trial shown here, the label "No Emotion" is presented for 3s, during which the participant is required to make a stereotyped monotonous vocalization, void of emotional content. A fixation cross is then presented for 5s.

Task 1 was designed as an event-related paradigm (Figure 8) to allow volumes containing significant motion artefacts to be excluded from analysis. A total of 72 trials were included in the task, with 36 trials from each condition. Trials belonging to the Emotion Condition were presented in groups of three, whereby the same type of emotion label was presented across trials (i.e., participants were expected to make three consecutive expressions of the same basic emotion, allowing them to become fully absorbed in the experience of producing the emotion). Trials belonging to the Neutral Condition were also presented in groups of three. Each group of three trials alternated between conditions. A total of 216 whole brain volumes were acquired for this fMRI run using a whole-brain T2*-weighted gradient-recalled echo-planar imaging sequence (EPI) with a TR of 3s.
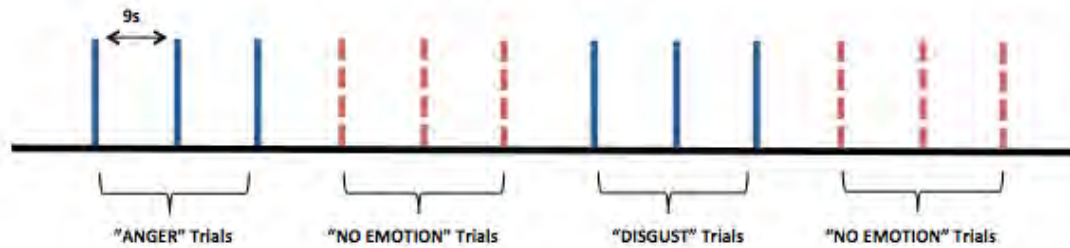

Figure 8. Series of events representing a sample of single trials from Task 1. Three trials from the Emotion Condition were presented consecutively, followed by three consecutive trials from the Neutral Condition. Across each group of three trials from the Emotion Condition, participants were expected to make emotional expressions belonging to the same emotion category (e.g., "Anger", "Disgust"). A fixed inter-stimulus interval of 9s was employed between trials.

Results to date show that the contrast of blood oxygenated level dependent (BOLD) signal acquired during the Emotion Condition versus Neutral Condition revealed several regions of significantly greater neuronal activation (t>3.16, p<0.001) associated with the production of vocal emotional expressions compared to non-emotional vocalizations. These regions include: (a) bilateral inferior frontal gyri, (b) bilateral superior frontal gyri (including premotor cortex, BA6), (c) bilateral superior temporal gyrus (including temporal pole, BA38), (d) left insula, (e) left anterior cingulate gyrus, (f) left parietal lobe, (g) right caudate, and (h) bilateral cerebellum. A number of these regions are displayed in Figure 9, which shows a single subject's BOLD activation map from Task 1.

Of note, our findings are consistent with other neuroimaging studies that have suggested that the regions of activation targeted in Task 1 underlie various emotion-related constructs. For example, limbic structures like the anterior cingulate cortex and insula have been associated with emotional processes that involve high cognitive demand (e.g., emotion recognition), the recall of emotional memories and emotional imagery (see Phan, Wager, Taylor, & Liberzon, 2002). In addition, the superior frontal gyrus, middle frontal gyrus and cerebellum have been implicated in studies on emotion regulation (Beauregard, Levesque, & Bourgouin, 2001; Eippert et al., 2007; Schutter & Honk, 2009). Further, the inferior frontal gyrus has been regarded as a voice-selective region that is particularly sensitive to nonverbal vocal emotional expressions (Fecteau, Armony, Joanette, & Belin, 2005). Interestingly, the inferior frontal gyrus—along with the superior temporal gyrus and parietal lobe also activated here—is a key structure in the MNS, which has important implications for understanding social cognition and empathy in the BAP (see Iacoboni & Dapretto, 2006), as discussed further in our General Discussion below.
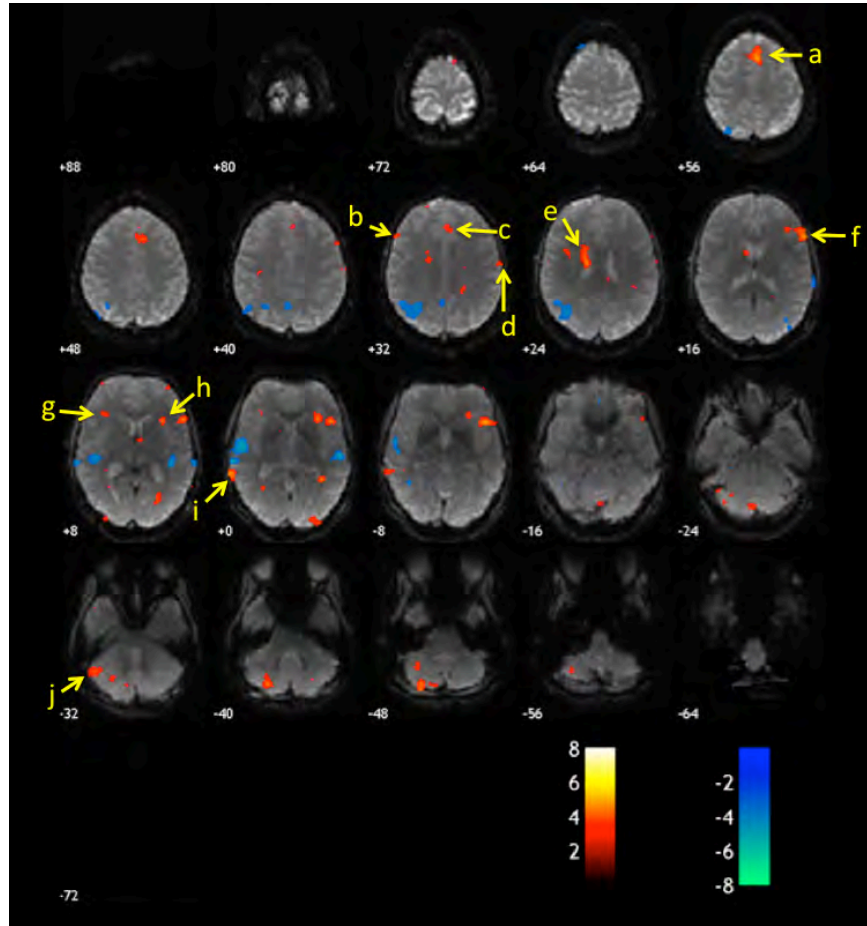
Figure 9. Activation map of axial slices from a single subject showing contrast in BOLD signal acquired during the Emotion Condition versus Neutral Condition in Task 1 (t>3.16, p<0.001). In this subject, several regions of significantly greater neuronal activation were associated with the production of vocal emotion expressions, including: a) superior frontal gyrus, b) right middle frontal gyrus, c) anterior cingulate, d) left post-central gyrus (parietal lobe), e) right caudate, f) left inferior frontal gyrus, g) left insula, h) right insula, i) right middle temporal gyrus, and j) right cerebellum.

Task 2: Vocal Emotion Recognition

In this task, participants listened to male and female nonverbal vocalizations belonging to two primary categories: (1) vocal expressions of the six basic emotions (Emotion Condition), and (2) non-emotional vocalizations (Neutral Condition). Figure 10 shows the sequence of events in the first trial from each condition. All vocal stimuli were taken from the Montreal Affective Voices (MAV). In the Emotion Condition, participants were required to identify the emotion in the speaker's voice by making a forced-choice discrimination between two emotion labels presented visually (e.g., "Anger" and "Disgust"). In the Neutral Condition, participants were required to identify the gender of the speaker by making a forced-choice discrimination between the labels "Male" and "Female" presented visually. Across both conditions, participants pressed a button box with their middle or index finger to indicate their response.
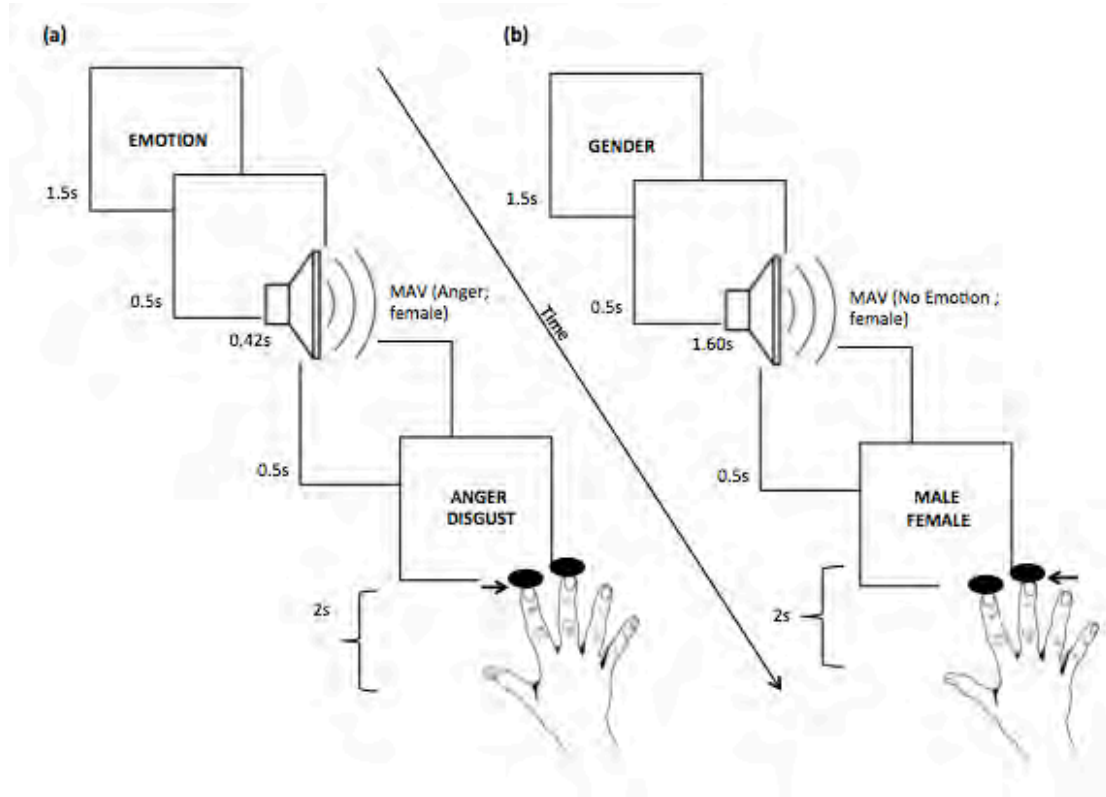
Figure 10. Sequence of events in the first trial of a block from the (a) Emotion Condition and (b) Neutral Condition in Task 2. At the onset of a block from the Emotion Condition, the label "Emotion" is presented for 1.5s. In the Emotion trial shown here, a MAV stimulus of an angry female voice is then presented (duration of MAV stimuli vary across trials). Next, the emotion labels "Anger" and "Disgust" are presented for 2s, during which the participant is required to select the emotion of the speaker by pressing a button with the index finger (for top label; correct response for this trial) or middle finger (for bottom label). At the onset of a block from the Neutral Condition, the label "Gender" is presented for 1.5s. In the Neutral trial shown here, a MAV stimulus of a non-emotional, monotonous female voice is then presented. The gender labels "Male" and "Female" are then presented for 2s, during which the participant is required to select the gender of the speaker by pressing a button with the index finger (for top label) or middle finger (for bottom label; correct response for this trial).

Task 2 was designed as an Epoch-based block paradigm (Figure 11). A total of 12 blocks were included in the task, with 6 blocks (6 trials per block) for each condition. The label "Emotion" was presented at the onset of each block from the Emotion Condition to alert participants to identify the speaker's emotion in the MAV stimuli presented across all trials within that block (Figure 10). Conversely, the label "Gender" was presented at the onset of each block from the Neutral Condition, to alert participants to identify the speaker's gender in the MAV stimuli presented across all trials within that block (Figure 10). Blocks were alternated between conditions, and trials within each block were presented in pseudo-randomized order. A total of 121 whole brain volumes were acquired for this fMRI run using a whole-brain T2*-weighted gradient-recalled EPI with a TR of 3s.
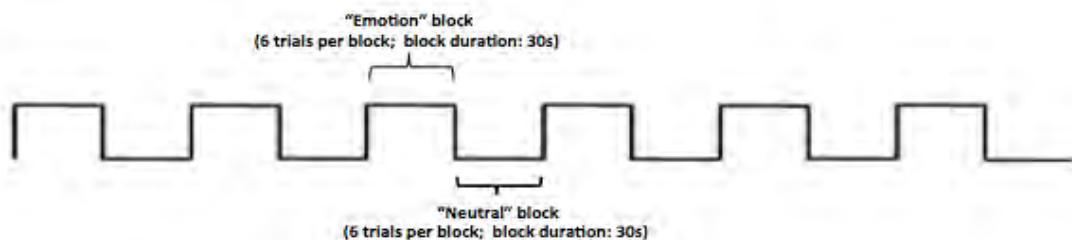

Figure 11. Classic "boxcar" model for Task 2 representing epochs of sustained activation for blocks from the Emotion Condition relative to blocks from the Neutral condition. Blocks for the Emotion Condition were alternated with blocks for the Neutral Condition, with 6 trials presented in each block over a 30s duration.

Our findings to date indicate that the contrast of BOLD signal acquired during the Emotion Condition versus the Neutral Condition revealed several regions of significantly greater neuronal activation ($t>3.17$, $p<0.001$) associated with the recognition of vocal emotional expressions compared to non-emotional vocalizations. Specifically, these regions include: (a) bilateral superior frontal gyri (including premotor cortex, BA6), (b) bilateral inferior frontal gyri, (c) bilateral middle frontal gyri, (d) bilateral superior temporal gyri (including temporal pole, BA38), (e) bilateral middle temporal gyri, (f) left parietal lobe (including supramarginal gyrus), and (g) bilateral cerebellum. A number of these regions are displayed in Figure 12, which shows a single subject's BOLD activation map for Task 2.

As for Task 1, the regions targeted by Task 2 also have significance for emotion processing. Further, it is interesting to note that common regions of activation were shared across Tasks 1 and 2, including structures in the MNS (i.e., inferior frontal gyrus, superior temporal gyrus, and parietal lobe). These findings suggest that shared neural mechanisms exist between the production and recognition of vocal emotional expressions, consistent with the findings from a previous study which showed activation in the inferior frontal gyrus (specifically) when participants passively listened to emotional vocal expressions and produced their own (Aziz-Zadeh, Sheng, & Gheytanchi, 2010). The implications of these findings are discussed in more detail below (see General Discussion).
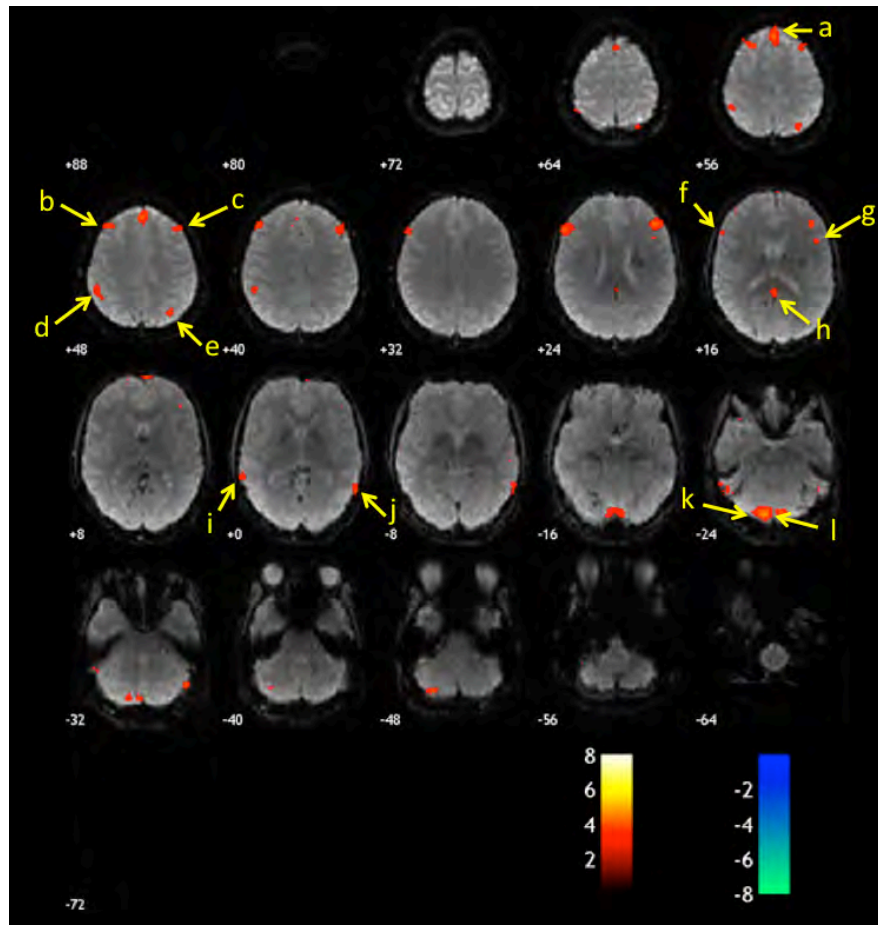

Figure 12. Activation map of axial slices from a single subject showing contrast in BOLD signal acquired during the Emotion Condition versus Neutral Condition in Task 2 ($t>3.17$, $p<0.001$). In this subject, several regions of significantly greater neuronal activation were associated with the recognition of vocal emotion expressions, including: a) superior frontal gyrus, b) right middle frontal gyrus, c) left middle frontal gyrus, d) right precuneus (parietal lobe), e) left supramarginal gyrus (parietal lobe), f) right inferior frontal gyrus, g) left inferior frontal gyrus, h) posterior cingulate, i) right middle temporal gyrus, j) left middle temporal gyrus, k) right cerebellum, l) left cerebellum.

<u>Tasks 1 and 2: General Discussion</u>

Our functional neuroimaging paradigm was designed to map brain systems that underpin social communication in the BAP. Specifically, we were interested in identifying systems that underlie the production and recognition of emotional vocal expressions, with the view of identifying neurobiological markers associated with the psychoacoustic endophenotype of the BAP.

Overall, our findings to date across Tasks 1 and 2 suggest that our functional neuroimaging paradigm has successfully targeted such systems, including limbic structures and the mirror neuron system (MNS), comprising premotor (e.g., inferior frontal gyrus), temporal (e.g., superior temporal gyrus) and parietal regions of the brain (Iacoboni & Dapretto, 2006). These findings represent an important scientific breakthrough, as it is the first time such a paradigm has been developed for use in individuals with the BAP. Previous research in healthy individuals has shown that the MNS includes a network of neurons that are commonly activated when individuals perform a specific action and when they observe others performing the same action (Rizzolatti & Craighero, 2004). Researchers have therefore proposed that the MNS underlies imitative behavior, which in turn, is crucial for the development of social skills such as understanding the intentions and emotions of other people (Carr, Iacoboni, Dubeau, Mazziotta, & Lenzi, 2003; Rizzolatti & Craighero, 2005). Accordingly, MNS dysfunction has been linked with imitation deficits, reduced empathy and greater symptom severity in ASD, and may thus be associated with similar features in the BAP (Dapretto et al., 2005; Hadjikhani, Joseph, Snyder, & Tager-Flusberg, 2005; Oberman & Ramachandran, 2007; Yamasaki et al., 2010).

The efficacy of our paradigm is further reflected in evidence for shared neural mechanisms between the production (Task 1) and recognition (Task 2) of emotions expressed vocally. These common regions of activation include structures belonging to the classic MNS network (i.e., inferior frontal gyrus, superior temporal cortex, parietal lobe) as well as other regions external to this network (i.e., superior frontal gyrus, middle temporal gyrus, insula, cerebellum). Of note, these latter regions have also been implicated in emotional processes, such as emotion regulation, emotion recognition, and emotional recall/imagery (Beauregard, et al., 2001; Eippert, et al., 2007; Phan, et al., 2002; Schutter & Honk, 2009). Our findings may therefore reflect simulation processes for emotional understanding, whereby individuals use internal representations of how they express vocal emotions to understand the vocal emotions expressed by others (Aziz-Zadeh, et al., 2010; Gallese, 2007). Accordingly, our neuroimaging paradigm provides an exciting new technique for examining differential patterns of activation during the production and recognition of vocal emotions in individuals with and without the BAP.

Finally, as described in our initial project proposal, examining MNS function in the BAP provides important insight into the neurobiological and genetic bases of social behavior in ASD. To this end, we are also working towards finalizing our analyses of larger sets of genetic, behavioral and functional neuroimaging data, which we will combine to provide conclusive evidence of MNS dysfunction in the BAP.

KEY RESEARCH ACCOMPLISHMENTS

The information below summarizes the key research accomplishments to date:

- IRB approval of study and successful ongoing IRB review of study across 2012-2014.

- Systematic development and pilot testing of the behavioral and neuroimaging protocols (Experiments 1 and 2), which includes the development of a new piece of computer software for the collection of the behavioral data, as well as the development of a novel fMRI protocol for the collection of in-scanner behavioral and brain imaging data.

- Ongoing training of the Research Assistant in paradigm development, data collection and data analysis for Experiments 1 and 2.

- Development of novel computational algorithms for analysis of voice recordings of BAP and control participants to characterize the psychoacoustic endophenotype of the BAP.

- Discovery of activation within the mirror neuron system, limbic system and other regions associated with vocal emotion processing, as revealed through analysis of our fMRI data

- Discovery of shared neural mechanisms between the production and recognition of nonverbal vocal emotional expressions.

- Detailed characterisation of the neurocognitive profile of the Broader Autism Phenotype (BAP) in family members of large families with multiple individuals affected with Autism Spectrum Disorders (ASD).

- Research presented at five scientific meetings between 2013-2014.

- Findings of Experiments 1 and 2 to be published in high impact, international scientific journals within the next 12 months.

- Presentation of findings from Experiments 1 and 2 to be disseminated at relevant community and scientific meetings over the next 12 months, including the International Meeting For Autism Research (IMFAR).

<u>CONCLUSION</u>

Overall, this project has been extremely successful and advanced the research field in a number of ways. First, it has led to the development of new online computer software to collect and analyze nonverbal vocal recordings sensitive to the BAP. These new techniques can now be applied to, and thus will facilitate the collection of large-scale behavioral data from individuals around the globe. Since such techniques have not previously existed, they represent a significant advance for the field. Second, the study has led to the development of a new neuroimaging protocol capable of assessing brain function and structure, particularly social and emotional functioning, in individuals with the BAP and ASD. Our findings to date indicate that our functional neuroimaging paradigm can be used to target regions within the mirror neuron system and limbic system of the brain, augmenting our understanding of the neurobiology of the BAP. Overall, such techniques had not previously been used with individuals with the BAP and thus, represent an important advance for the field. Combined, the techniques now provide new and exciting ways for objectively assessing core features of autism that have eluded the field to date. This will ultimately provide important new avenues for identifying genes underpinning ASD and the BAP.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

Preliminary aspects of the research was presented at five scientific meetings between 2013-2014, as follows:

1) *Verbal Pragmatics and Vocal Emotion Processing in the Broader Autism Phenotype.* Oral presentation, Neurocognition & Neuroimaging Laboratory, The University of Melbourne (March, 2013).

2) *Verbal Pragmatics and Vocal Emotion Processing in the Broader Autism Phenotype.* Oral presentation, Collaborative Autism Team Study, Annual Autism Retreat, The University of Melbourne. This retreat is well attended by researchers from The University of Melbourne, the Royal Children's Hospital, the Melbourne Brain Centre, and the Florey Institute of Neuroscience and Mental Health (May, 2013 and November 2014).

3) *Verbal Pragmatics and Vocal Emotion Processing in the Broader Autism Phenotype.* Platform presentation, Neurocognition & Neuroimaging Workshop, 2013 Epilepsy Research Retreat, Melbourne Brain Centre, Florey Institute of Neuroscience & Mental Health, The University of Melbourne, Australia (July 2013).

4) *Vocal Emotion Processing in the Broader Autism Phenotype.* Platform presentation, School of Psychological Sciences, The University of Melbourne, Australia (November 2014).

INVENTIONS, PATENTS, AND LICENSES

Nothing to report.

REPORTABLE OUTCOMES

- Development of a novel web-based paradigm to collect extensive data sets of vocal responses across tasks of spontaneous production, recognition and imitation of nonverbal emotional vocal expressions.

- Development of a novel functional neuroimaging paradigm to target the mirror neuron system and other brain regions of significance to social and emotional processing in the BAP and ASD.

OTHER ACHIEVEMENTS

Nothing to report.

REFERENCES

Aziz-Zadeh, L., Sheng, T., & Gheytanchi, A. (2010). Common Premotor Regions for the Perception and Production of Prosody and Correlations with Empathy and Prosodic Ability. *PLoS One, 5*(1), e8759.

Beauregard, M., Levesque, J., & Bourgouin, P. (2001). Neural Correlates of Conscious Self-Regulation of Emotion. *The Journal of Neuroscience, 21*, 1-6.

Belin, P., Fillion-Bilodeau, S., & Gosselin, F. (2008). The Montreal Affective Voices: A validated set of nonverbal affect bursts for researchon auditory affective processing. *Behavior Research Methods, 40*(2), 531-539.

Carr, L., Iacoboni, M., Dubeau, M.-C., Mazziotta, J. C., & Lenzi, G. L. (2003). Neural mechanisms of empathy in humans: A relay from neural systems for imitation to limbic areas. *Proceedings of the National Academy of Sciences, 100*(9), 5497-5502.

Dapretto, M., Davies, M. S., Pfeifer, J. H., Scott, A. A., Sigman, M., Bookheimer, S. Y., & Iacoboni, M. (2005). Understanding emotions in others: mirror neuron dysfuntion in children with autism spectrum disorders. *Nautre Neuroscience, 9*(1), 28-30.

Eippert, F., Veit, R., Weiskopf, N., Erb, M., Birbaumer, N., & Anders, S. (2007). Regulation of Emotional Responses Elicited by Threat-Related Stimuli. *Human Brain Mapping, 28*, 409-423.

Fecteau, S., Armony, J. L., Joanette, Y., & Belin, P. (2005). Sensitivity to Voice in Human Prefrontal Cortex. *Journal of Neurophysiology, 94*, 2251-2254.

Gallese, V. (2007). Before and below 'theory of mind': embodied simulation and the neural correlates of social cognition. *Philosophical Transactions of the Royal Society B, 362*, 659-669.

Hadjikhani, N., Joseph, R. M., Snyder, J., & Tager-Flusberg, H. (2005). Anatomical Differences in the Mirror Neuron System and Social Cognition Network in Autism. *Cerebral Cortex, 16*(9), 1276-1282.

Iacoboni, M., & Dapretto, M. (2006). The mirror neuron system and the consequences of its dysfunction. *Nature Reviews Neuroscience, 7*, 942-951.

Oberman, L. M., & Ramachandran, V. S. (2007). The Simulating Social Mind: The Role of the Mirror Neuron System and Simulation in the Social and Communicative Deficits of Autism Spectrum Disorders. *Psychological Bulletin, 133*(2), 310-327.

Phan, K. L., Wager, T., Taylor, S. F., & Liberzon, I. (2002). Functional Neuroanatomy of Emotion: A Meta-Analysis of Emotion Activation Studies in PET and fMRI *NeuroImage, 16*(2), 331-348.

Rizzolatti, G., & Craighero, L. (2004). The Mirror-Neuron System. *Annual Review of Neuroscience 27*, 169-192.

Rizzolatti, G., & Craighero, L. (2005). Mirror neuron: a neurological approach to empathy. In J.-P. Changeux, A. R. Damasio, W. Singer & Y. Christen (Eds.), *Neurobiology of Human Values* (pp. 107-123): Springer Berlin Heidelberg.

Schutter, D., & Honk, J. v. (2009). The Cerebellum in Emotion Regulation: A Repetitive Transcranial Magnetic Stimulation Study. *The Cerebellum, 8*(1), 28-34.

Yamasaki, S., Yamasue, H., Abe, O., Suga, M., Yamada, H., Inoue, H., . . . Kasai, K. (2010). Reduced Gray Matter Volume of Pars Opercularis Is Associated with Impaired Social Communication in High-Functioning Autism Spectrum Disorders. *Biological Psychiatry, 68*, 1141-1147.